The relation of tear staining to growth, sex and potential stress factors in finisher pigs

Anna Gustafsson

Licentiate thesis in veterinary medicine

Department of Production Animal Medicine

Faculty of Veterinary Medicine

University of Helsinki

2020

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta - Fakultet - Faculty Eläinlääketieteellinen tiedekunta	Osasto - Avdelning – Department Kliinisen tuotantoeläinlääketieteen osasto	
Tekijä - Författare - Author Anna Gustafsson		
Työn nimi - Arbetets titel - Title The relation of tear staining to growth, sex and potential stress factors in finisher pigs		
Oppiaine - Läroämne - Subject Tuotantoeläinten hyvinvointi		
Työn laji - Arbetets art - Level Lisensiaatin tutkielma	Aika - Datum - Month and year 1/2020	Sivumäärä - Sidoantal - Number of pages 36

Tiivistelmä - Referat – Abstract

Sikojen tummaa kyynelvuotoa on aiemmin pidetty lähinnä merkkinä kliinisestä sairaudesta tai huonosta ilmanlaadusta. Viimeaikaisissa tutkimuksissa vuoto on kuitenkin yhdistetty myös erilaisiin stressitekijöihin. Mikäli kyynelvuodon määrä kertoo eläimen kokemasta stressistä, voisi se olla hyödyllinen apuväline arvioitaessa sikojen hyvinvointia tilaolosuhteissa. Silmävuodon käyttöä hyvinvoinnin arvioinnin apuvälineenä on aiemmin käytetty yleisesti rotilla, joilla nopeasti kehittyvän punaisen kyynelvuodon aiheuttaa Harderin rauhasen aktivaatio. Sama rauhanen löytyy myös sioilta, joten on mahdollista, että ilmiön taustalla on samankaltainen mekanismi molemmilla lajeilla.

Tämän lisensiaatintyön kirjallisessa osassa kuvattiin sikojen kyynelvuotoon liittyvät anatomiset ja fysiologiset tekijät sekä nykytietämys silmävuodon yhteydestä stressiin. Työn kokeellisessa osassa tutkittiin lihasikojen silmävuodon kehittymistä tilaolosuhteissa sekä sen yhteyttä ikään, kasvuun, sukupuoleen ja kokeellisesti aiheutettuihin stressitekijöihin. Tutkimus tehtiin kolmessa erässä, yhteensä mukana oli 80 karsinaa ja 1160 sikaa. Kussakin erässä eläimet jaettiin tutkimuskarsinoihin hännän tyypin (typistetyt hännät tai typistämättömät hännät), virikemateriaalitarjonnan (150 g olkea päivittäin eläintä kohti tai ei lainkaan olkea) sekä elintilan (1,21 m2 tai 0,73 m2 tilaa eläintä kohti) mukaan. Kyynelvuodon määrä pisteytettiin yksilöllisesti erikseen kummastakin silmästä asteikolla 1-4 pienemmästä vuodosta suurempaan, kolme kertaa viikossa yhdeksän viikon ajan. Samalla arvioitiin myös eläinten häntävauriot. Jokaisella tutkimusviikoilta valittiin tilastoanalyysiin kultakin yksilöltä korkein havaittu pisteluku. Kerätty data analysoitiin käyttäen logistista regressiomallia erikseen jokaiselle neljälle pisteluvulle.

Pisteiden 1 ja 2 määrät vähenivät tutkimusjakson aikana ja korreloivat negatiiviseti keskimääräisen päiväkasvun kanssa. Pisteiden 4 määrä sen sijaan kasvoi tutkimusjakson aikana ja korreloi positiivisesti keskimääräisen päiväkasvun kanssa. Sukupuolella, oljen tarjoamisella tai elintilalla ei ollut vaikutusta pisteisiin. Tutkimuksessa pisteiden 1 määrä väheni ja pisteiden 4 määrä nousi selkeän häntävaurion ilmenemistä edeltävän viikon aikana. Tulokset viittaavat kyynelvuodolla olevan yhteys sian ikään, kasvuun ja koettuun stressiin. Elintilalla tai oljen tarjoamisella ei ollut tutkimuksessa vaikutusta kyynelvuotoon. Mikäli kyynelvuotoa halutaan käyttää sikojen hyvinvoinnin arvioinnissa, tulee huomioida iän ja kasvun vaikutus vuodon määrään.

Avainsanat - Nyckelord - Keywords

silmävuoto, kyynelvuoto, sika, lihasika, harderin rauhanen, stressi, hännänpurenta

Säilytyspaikka - Förvaringställe - Where deposited

HELDA – Helsingin yliopiston digitaalinen arkisto

Työn johtaja (tiedekunnan professori tai dosentti) ja ohjaaja(t) - Instruktör och ledare - Director and Supervisor(s)

Johtaja ja ohjaaja professori Anna Valros
Ohjaaja Mona Lilian Vestbjerg Larsen, PhD, Aarhus University

CONTENTS

1 INTRODUCTION

Staining around the inner corners of the eyes is a common occurrence in most domestic species. Common causes for this phenomenon are chronic overproduction of tear fluid due to inflammation of the tissues of the eye and blockage of the tear fluid drainage system (Glenwood & MacKay 2013). Not all eye discharge is linked to clinical disease: Rats exhibit a distinctive type of tear staining (TS), known as chromodacryorrhoea, meaning secretion of bright red tear fluid (Evans & Donnelly 2013). The connection between chromodacryorrhoea and stress has been well established in laboratory rats and chromodacryorrhoea has been used as an indicator of compromised welfare in that setting (Baumans 2004). The colour is caused by porphyrins, compounds produced by an orbital gland found in most mammals, the Harderian gland (HG) (Payne 1994, review). Pigs also have a HG that produces porphyrins (McCafferty & Pinkstaff 1970) and they show TS. staining. Recently, it has been suggested that TS could also be an indicator of stress in pigs (DeBoer et al. 2015, Telkänranta et al. 2016). Significant environmental stressors in the modern pig production include high stocking density and lack of environmental enrichment (Martínez-Miró et al. 2016, review). Lack of possible coping strategies for the pig in the commercial farm environment can lead to tail biting behaviour, which is therefore both a consequence and a source of stress for the pig (Taylor et al. 2010, review). In addition to being a welfare problem, tail damage is a cause for economic losses in the form of slower growth rate (Sinisalo et al. 2012) and increased numbers of carcass condemnations due to infections (Valros et al. 2004). Strategies for preventing tail biting behaviour include removing or diminishing known stressors or identifying the situations where the risk for tail biting behaviour is increased and intervening before significant tail damage occurs (Larsen et al. 2018a, Larsen et al. 2018b). Thus, there is a need for finding indicators of increased stress in pigs. Ideally, evaluating the indicators should be time and cost efficient to be applicable in the commercial production farm. Therefore, TS would be a potential candidate, if it proves to increase (or decrease) in relation to tail biting.

The purpose of this thesis was to first describe the anatomical and physiological features behind TS in pigs as well as investigate what is known about the connection of TS and stress in domestic animals in general; this will be covered in the literature review in

Section 2. Second, the aim of the experimental part was to determine how TS in finishing pigs develops in farm conditions during the slaughter pig period and to investigate its relation to gender, pen-level stressors and average daily gain (ADG). The TS of the left and the right eye were studied separately. The pen-level development of TS prior to occurrence of significant tail damage was also investigated to explore the potential of TS for being an early detector of tail biting damage. The experimental part will be covered in Section 3 to 6.

2 LITERATURE REVIEW

2.1 Tear production

2.1.1 The nasolacrimal apparatus

The nasolacrimal apparatus is an accessory system to the eye and includes the various species-specific glandular structures that produce the tear fluid as well as the structures that allow the drainage of the fluid into the nasal cavity. Other major accessory structures that protect the eye and enable its function are the eyelids and eyelashes. The eyelids cover the eye during sleep and blinking, protect the eye from excessive radiation, irritating chemicals and foreign objects as well as prevent the surface from dehydration and assist with lubricating the eye (Akers & Denbow 2013). The main function of the eyelashes is to help keep foreign objects and direct sunlight out of the eye (Akers & Denbow 2013).

The lacrimal gland synthesizes and secretes the majority of the volume of tear film (Glenwood & MacKay 2013). The lacrimal glands in pigs, similarly to humans, are located dorsolaterally in relation to the eyeball, in the upper temporal portion of the orbit (Henker et al. 2013). Histologically, they are multilobar with acini composed of large serous cells filled with secretory granules and surrounded by myoepithelial cells (Junquira & Carneiro 2003). The lacrimal glands in the pig drain through several excretory ducts opening into the recess between the upper eyelid and the eye (Henker et al. 2013). The tear fluid covers the surface of the eye and drains into the lacrimal puncta, small openings on the upper and lower eyelid near the medial corner of the eye (Akers &

Denbow 2013). There is some variation in the number of lacrimal puncta between species: Rabbits have only a single punctum on the lower eyelid (Rehorek et al. 2011) and camels do not have lacrimal puncta at all (Ibrahim et al. 2006). Pigs have a single functional upper lacrimal punctum (Gelatt 2011). From the puncta the fluid enters the lacrimal canals, leading into the lacrimal sac. The fluid is then carried into the nasal cavity through the nasolacrimal duct, where it provides moisture to the nasal mucosa (Akers & Denbow 2013).

In addition to the lacrimal gland, there are several other glands contributing to the production of the tear fluid. Embedded in the margin of both upper and lower eyelids is a row of sebaceous glands called the meibomian glands, and together with the sebaceous glands of eyelash hair follicles they secrete fluid that helps prevent the eyelids from adhering from one another (Akers & Denbow 2013), creating the lipid component of the tear fluid (Dartt 2004). Domestic species have a structure that humans lack: the nictitating membrane, also known as the third eyelid, which arises from the inner corner of the eye. Closely attached to its base is a glandular structure, a sebaceous gland of the third eyelid, which can be further divided into a deep and a superficial part. The deep gland is also called the Harderian gland and is described in detail in Section 2.2. Some species, such as the dog, only have the superficial part of the gland (Cabral et al. 2005). However, some embryologic and histochemical studies suggest that making a strict distinction between the superficial and deep glands of the third eyelid is perhaps not justified. In an embryologic study in deer, it was discovered that the superficial and deep glands of the third eyelid originate from the same glandular primordium (Rehorek et al. 2007). Further evidence on close relationships between the different orbital glands is the phenomenon of harderianization: the age-related appearance of structures resembling the HG in the lacrimal glands of aging male rats (Gancharova & Manskikh 2014).

2.1.2 Tear fluid and the lacrimal film

The lacrimal fluid moistens and lubricates the cornea and conjunctiva and provides oxygen to the corneal epithelial cells (Junquira & Carneiro 2003). There are three layers in the tear film. The superficial layer consists of lipids from the meibomian glands, the

aqueous layer is produced by the lacrimal gland and the glands of the third eyelid, and the innermost layer is produced by the secretory cells of mucosa (Dartt, 2004). The superficial layer provides lubrication to the eyeball, prevents overflow of tear fluid from the lid margin and retards evaporation of the underlying aqueous layer. The aqueous layer contains various metabolites, electrolytes, and proteins of innate immunity such as antibodies and lysozyme (Junquira & Carneiro 2003). The mucous layer further provides a physical and chemical protective barrier between the surface of the eye and the surroundings (Dartt 2004).

2.1.3 Regulation of lacrimation

The secretions of water, electrolytes and proteins from the lacrimal gland are neurally controlled, which allows for a fast response to changing environmental conditions (Dartt 2009, review). The lacrimal glands produce a basal rate of lacrimal fluid, but lacrimation can increase in response to many types of stimuli, including changes in humidity, light, temperature or sensation of pain or irritation. This is known as reflexive lacrimation (Meng & Kurose 2013). Humans can also exhibit increased lacrimation in response to changes in the emotional state, resulting in the everyday phenomenon of crying. This has been considered a uniquely human trait.

Reflexive lacrimation is caused when the neural receptors in the conjunctiva and cornea of the eye are activated. There are three main types of receptors: mechanoreceptors that are sensitive only to mechanical stimuli, polymodal receptors that respond to thermal, chemical and mechanical stimulation, and cold receptors, which are activated by non-pathologic cooling associated with normal evaporation of tear fluid from the surface of the eye (Meng & Kurose 2013). Reflexive tearing is the result of activation of the efferent nerves controlling the secretory cells and excretory ducts of the lacrimal gland (Dartt 2009, review). The parasympathetic fibres cause increased secretion of water, electrolytes and proteins while the sympathetic nerves control the blood flow into the gland, which affects tear fluid production rate (Remington 2012). Lacrimation, however, is not merely a simple reflex, as the sensory input is processed in the lacrimal nucleus of the brain, together with other input from other centers to generate a graded output (Meng & Kurose 2013).

2.2 The Harderian gland

2.2.1 Anatomy and development

The Harderian gland (HG) was first described by Harder in 1694 in deer (Payne 1994, review). Humans do not have HGs, which might be the reason why relatively little is still known about this organ and its functions. The gland has been described in many domestic species including the domestic duck (Oliveira et al. 2006), rat (Ortiz et al. 2001 and Sbarbati et al. 2002), mouse (Ortiz et al. 2001), Syrian hamster (Ortiz et al. 2001), guinea pig (Ortiz et al. 2001), sheep (Abd et al. 2014), cattle (Pinard et al. 2003) and the domestic pig (Munkeby et al. 2006). Apparently, it is absent in some mammals, such as horses and terrestrial carnivores, including dogs and cats (Payne 1994, review; Cabral et al. 2005). The HG in mammals is located in the orbit, ventromedially to the eyeball, with the duct opening on the surface of the third eyelid (Chieffi et al. 1996, review). In the neonatal pig, it can be relatively easily distinguished in dissection from the surrounding structures as it forms a distinct mass (Munkeby et al. 2006).

In the domestic pig, the anatomy of the HG has been described in detail only in the new-born piglet. Munkeby et al. (2006) examined the gland in piglets with magnetic resonance imaging, dissection and histology and found that in the piglet the gland is spherical in shape, extends from the cartilage of the nictitating membrane towards the bottom of the orbit and has the diameter of about half of the relative eyeball. The gland is also partially surrounded by vascular sinuses, bordering medially on the olfactory bulbs and ethmoidal conchae. However, the postnatal development of the pig HG has so far not been described. From studies on the postnatal development of the HG in guinea pigs and Syrian hamsters we know that the glands of these species go through several morphological changes in the first few weeks after birth (López et al. 1992; Elgayar et al. 2015). The gland seems to be immature in structure at birth and reach the structural characteristics of adult glands by the age of three weeks in the guinea pig and seven weeks in the hamster (López et al., 1992; Elgayar et al. 2015). For the Syrian hamster, sex differences begin to appear at the age of 20 days (López et al. 1992). After maturation the gland continues to grow in size,

in the hamster up to the age of two months (Elgayar et al. 2015; López et al. 1992). Histologically, the HG in mammals consists of tubular or tubuloacinar alveoli and myoepithelial cells (Payne 1994 review; Chieffi et al. 1996, review). In this sense the HG of the new-born pig is typical among mammals. It has a lobular structure, tubuloacinar cells with numerous eosinophilic granules, a fine capillary network and sparse connective tissue (Munkeby et al. 2006). Thus, the pig HG may also go through similar morphological changes with age.

2.2.2. Secretory activity

The glandular cells of HG have been found to secrete many different compounds, with major groups being lipids, indoles and porphyrins (Payne 1994, review). Lipids are the predominant secretory product in mammals, and porphyrins, usually found as solid accretions in the lumina of the gland, are apparently only secreted by mammalian HGs (Payne 1994, review; Chieffi et al. 1996, review). Porphyrins are a group of organic compounds, chemically characterized by a tetrapyrrolic ring, which appear dark red for the human eye in visible wavelengths of light and fluoresce under UV light. The heme group found in haemoglobin in the red blood cells is the best-known porphyrin, but there are many other biologically relevant porphyrins as well. The synthesis of porphyrin in the HG has been extensively studied in rodents. It seems that in rats, mice and hamsters, the porphyrin content of HGs is quite low at birth and starts rapidly increasing after the first week of age (Chieffi et al. 1996, review). Porphyrin levels in the rat HG increase up to 20 months and decreases in 24-month-old rats, but food restriction prevents the age-associated rise (Rodríguez et al. 1992). In addition to porphyrins, the various lipids and indoles found in the HG have also been a subject of interest (Payne 1994, review). However, the exact functions of these compounds still remain largely hypothetical. Some suggestions about the functions of the HG include lubrication for the nictitating membrane and the eye, immunoresponse, photoprotection and –reception and social signalling through pheromone production (Payne 1994, review). The exact compounds in the pig HG secretions have not been studied in detail.

2.2.3. Sexual dimorphism

In some species, such as the guinea pig, Syrian hamster and miniature pig, the HG has been found to exhibit sexual dimorphism (McCafferty & Pinkstaff 1970; Buzzell 1996; Hussein et al. 2015). This means that in these species some features of the gland, such as cell structures or patterns of secretion are markedly different in male and female animals. This in turn suggests that the gland, at least in some species, is regulated by sex steroid hormones. The sexual dimorphism is especially notable in the Syrian hamster, where the morphological differences are observable after the first month of age, which coincides with a rise in testosterone plasma levels (Rodríguez-Colunga et al. 1993). The difference is manifested by markedly higher porphyrin levels in female glands as well as morphologically different secretory cells. Female Syrian hamster secretory cells have typically only small lipid vacuoles while the male glands have additional populations of secretory cells with large lipid vacuoles (Buzzell 1996). In short, it seems that the different types of lipid droplets in the secretory cells, as well as the amount of porphyrin in the gland, are modified by androgen levels (Buzzell 1996). As a result, the castration of male hamsters and subsequent drop in the testosterone levels leads to feminization of the morphology and porphyrin levels of the HG and the administration of testosterone to adult female hamsters results in a marked increase in the percentage of more male-like secretory cells in the HG (Buzzell 1996).

The miniature pig HG also shows marked sexual dimorphism (McCafferty & Pinkstaff 1970). Structural dimorphism can be seen regardless of age, and differences in histological staining of the HG begin to appear at the age of two months (McCafferty & Pinkstaff 1970). After this point, the female glands possess higher concentrations purple-staining substance (in Alcian blue-periodic acid-Schiff staining) in contrast to reddish staining in the male glands (McCafferty & Pinkstaff 1970). It is specifically this purple staining substance in the female pig HG that is believed to be porphyrin (McCafferty & Pinkstaff 1970).

2.2.4 Regulation

Studies in the hamster indicate that the blood vessels, myoepithelial cells, and gland epithelium are all richly innervated in the HG, but the nerves are not present in the neonate (Chieffi et al. 1996, review). The finding that rats given acetylcholine produce porphyrin-

rich lacrimation is highly suggestive of a cholinergic action on gland contractility (Harkness & Ridgway 1980). Photoperiod and temperature seem to be the main extrinsic cues suspected to be involved in the control of HG physiology, as both affect the N-acetyltransferase activity and melatonin content of the hamster HG (Chieffi et al. 1996, review).

In addition to melatonin and androgens, there is evidence of hormonal regulation of the HG by the thyroid hormones in particular. Triiodothyronine (T3) and its prohormone thyroxin (T4) have been a topic of interest in many studies (Chieffi et al. 1996, review; Chieffi et al. 2004). Long-term changes in the levels of thyroid hormones in plasma significantly and directly affect porphyrin concentrations and morphology in the HGs of hamsters (Hoffman et al. 1990). In the rat, it is shown that hypothyroidism results in reduced lipid secretion in the HG (Monteforte et al. 2008) while the administration of T3 increases the mitochondrial resipratory activity and consequently induces lipogenesis and the release of secretory products in the HG (Baccari et al. 2004). There is also evidence that some pituitary hormones, such as prolactin, regulate the porphyrin synthesis of the HG in hamsters (Payne 1990).

2.3 Tear staining and chromodacryorrhoea

2.3.1 Causes of tear staining

Observable tear staining (TS) is caused by overflow of tear fluid over the eyelid margins. When the secretion rate of exocrine orbital glands exceeds the normal drainage capacity of the nasolacrimal duct, or the drainage is disrupted, the clinical signs of excessive tearing, also known as epiphora, can be detected (Sjaastad et al. 2010, Gelatt 2011). Excessive lacrimation can be stimulated by many different stimuli from mechanical and chemical irritation of the eye to changes in the humidity or temperature of the surroundings. Many pathological conditions of the nasolacrimal apparatus and infectious diseases of the eye or nasal passages can also cause secretions of various colours and consistencies (Glenwood & MacKay 2013). Bloody nasal or ocular discharge in domestic animals is possible as well. This is not a very common condition but could be caused by such underlying reasons as a trauma to the eye or surrounding tissues or irritation of the

nasal mucosa. In literature, TS in pigs is mentioned as a clinical sign of many infectious diseases, including Aujezsky's disease, Classical swine fever, *Bordetella bronchiseptica* rhinitis and atrophic rhinitis, as well as blocked tear ducts in general (Done et al. 2012, Duncanson 2013). Irritating gases, especially ammonia in large quantities have also been associated with TS in pigs (Drummond et al. 1980, Done et al. 2012). Common pathological causes for TS in other domestic species include foreign bodies, allergic reactions, infections in one or more structures of the eye, glaucoma, the dry-eye syndrome and blockage of the lacrimal duct (Gelatt et al. 2013).

As there are many possible causes for excess lacrimation, it could be that different stimuli trigger varying responses from different glands. Dark colour of a stain could be an indicator of high porphyrin content, produced by the HG, but environmental factors, such as dust and dirt could affect the colour of the stains. High production rate of lacrimal fluid could also possibly dilute the darker HG secretions. Mason et al. (2004) suggest that, because porphyrins secreted by the HG fluoresce under UV light, a so-called Wood's lamp might be useful in detecting the secretions. Presence of fluorescing substances in the tear stain is not necessarily a sign of HG activation, since there are other sources of fluorescing biological porphyrins as well, such as the aforementioned haemoglobin in red blood cells.

2.3.2. Chromodacryorrhoea

Some rodent species, most notably the laboratory rat, exhibit a specific type of TS called chromodacryorrhoea, colloquially known as red tears (Mason et al. 2004). This means characteristic red tear fluid appearing around the eyes and the nose (Harkness & Ridgway 1980). The reddish-brown eye discharge can be secreted copiously and is therefore easily observable on the face of the animal. The colour of the secretion is a result of presence of porphyrins, secreted in the tear fluid by the HG (Payne 1994, review). In rodents it is also known that, in addition to mere secretion, porphyrins are also synthetized in the HG (Buzzell 1996). This may indicate that the same is true for other species that secrete porphyrins from their HGs.

In the 1980 study by Harkness and Ridgway, chromodacryorrhoea in rats could be observed several minutes after the animal was exposed to a strong stressor and the lacrimation lasted for approximately two hours. On average, larger rats had a latent period between stressor and chromodacryorrhoea of 17 minutes, medium sized rats had a latent period of 22 minutes and small rats had a period of 30 minutes. This seems to show a general tendency towards larger rats developing chromodacryorrhoea quicker, but the sample size in the study was rather limited (15, 20 and six rats in the large, medium and small size groups, respectively). The development of observable chromodacryorrhoea could also be partly attributed to a decrease in grooming behaviour, which coincides with painful or otherwise stressful situations and allows the secretions to accumulate around the eyes and the nose (Mason et al. 2004).

2.3.3 Tear staining and stress

Chromodacryorrhoea has been widely used as an indicator of acute stress and discomfort in laboratory rodents (Baumans 2004). This is a practical assessment tool, as the dark stains are easily discernible in light coloured laboratory rodents and can be observed without handling the animal. In rat studies it has been associated with many different stressors such as limb restraint (Harkness & Ridgway 1980), temporomandibular joint pain and inflammation (Kerins et al. 2002), strong aversive odours (Burn et al., 2008) as well as milder environmental stressors, such as noise, moving of the rats' cage or unknown humans visiting in the laboratory space where the cages are kept (Mason et al. 2004). Harkness & Ridgway (1980) found out that injected acetylcholine elicits almost immediate chromodacryorrhoea, while the restraint of the forelimbs also leads to tear staining but with a latent period of several minutes. Furthermore, a treatment with atropine (an anticholinergic agent) blocked the development of tear staining. Similarly, Santos & Carlini (1988) observed that chromodacryorrhoea was induced in rats that were treated with pilocarpine, oxotremorine and neostigmine while administration of anticholinergics inhibited the effect. This suggests that chromodacryorrhoea in rats is indeed a result of a systemic response to a stressor. In the study by Harkness & Ridgway (1980) water and food deprivation and temperature stress did not induce chromodacryorrhoea, which implies that it might perhaps be triggered by only the more acute kind of stressors or by a stressor against which the rat can poorly cope. Mason et al.

(2004) studied the effect of milder environmental stressors such as construction noise to laboratory rats and found out that these also elicit chromodacryorrhoea and that the extent of staining around the nose is correlated with the intensity of the stressor. In none of the aforementioned studies were any sex or left vs. right eye differences reported.

The idea that tear staining could be a direct stress indicator also in pigs, rather than an indicator of underlying clinical disease, is rather recent and consequently there are few published studies about the topic. To date it is also unclear whether the physiology of TS in pigs is indeed similar to the one of chromodacryorrhoea in rodents. In 2007 Whay et al. observed tear staining in commercial finishing pig units in the UK as part of collecting preliminary data for assessment of pig welfare using animal-based indicators. Tear staining was assessed together with bodily lesions such as tail, flank and ear lesions, soiling and lameness. They found that the overall prevalence of tear staining in the study was 62.2%, but do not comment on how tear staining was defined by the observers. In a 2002 study (Gruber 2002 in Whay et al. 2007), the prevalence of tear staining in Austrian finishing pigs was 6%. The remarkable difference in the numbers might suggest that the studies had different definitions for tear staining.

The first reported study on the subject of pig tear staining and stress was conducted by DeBoer et al. (2015), who studied the effects of isolation and barren environment on the tear staining of young fattening pigs in laboratory conditions. The pigs received four different treatments: 1) isolation and barren environment, 2) a visual companion and barren environment 3) isolation and slightly enriched environment and 4) a visual companion and slightly enriched environment. They found that tear staining was most extensive in the isolation and barren environment group, which suggests that tear staining could be a sign of stress in pigs as well. Further studies suggest that tear staining in pigs is also correlated to measures of HPA and SAM axis activation (DeBoer & Marchant-Forde 2013). Telkänranta et al. (2016) conducted a study under commercial farm conditions to test the validity of tear staining as a welfare indicator on farm. They found significant correlations between individual tear staining scores and tail or ear damage, suggesting that tear staining could be a consequence of the systemic response to the damage-induced pain or related to the stress the injured individual has evidently undergone. The pen-level tear staining scores were negatively correlated to the quality of the manipulable objects in the pen, meaning that the pens that had access to more interesting objects had lower tear staining scores. They also noted that within-pen

variation of the scores was very high, and that the scores were quite low in suckling piglets and appeared to increase with age. Enrichment for suckling piglets interestingly lowered the lactating sow's tear staining scores, even though the enrichment material was not accessible to the sow.

A difference in tear staining between the left and the right eye in pigs has been reported by Telkänranta et al. (2016) and DeBoer et al. (2015). In both studies, the size of the left eye tear stains appeared to correlate more with assumed stress factors. This is suggested by Telkänranta et al. (2016) to be the result of cerebral lateralisation, a phenomenon where the left and right brain hemispheres process information differently, which may lead to animals being more reactive to threats in the left-eye visual field (Leliveld et al. 2013). The timeline of the development of TS in pigs has so far not been recorded very precisely, but it is known that TS can increase significantly within 24 hours (DeBoer et al. 2015).

Mason et al. (2004) found that in rats a low social status in the form of submissiveness in food competition situations was correlated with higher chromodacryorrhoea scores when exposed to environmental stressors on an unrelated occasion. A possible explanation, if we accept the hypothesis that higher TS scores indicate higher stress, is that submissive animals are more easily stressed in general and therefore their systemic response to environmental and social stressors is stronger than the dominant individuals' response. A similar finding with pigs was reported by Marchant-Forde and Marchant-Forde (2014), who recorded weaner pigs and analyzed their aggressive interactions, determining the winner and loser of each such interaction. From this data the social rank of each pen was calculated. It was found that the extent of TS of the left eye correlated with lower social rank, indicating that the amount of TS around the left eye could be a measure of social stress as well. In the 2016 study of Telkänranta et al. longer latency in approaching a novel object or human, perhaps indicating a more fearful mental state, was also associated with higher tear staining scores. However, fearfulness in a novel object test and fearfulness in a human approach test have showed weak correlations in pigs, suggesting each test could be measuring different personality traits, not overall proneness to stress (O'Malley et al. 2019). It is therefore not straightforward to draw a connection between overall fearfulness and tear staining in pigs.

2.4 Pigs and stress in the commercial farm environment

2.4.1 Different types of stress

Intensively housed pigs experience various types of stressors. Social stress stems from the interactions with other animals or social isolation. In commercially farmed pigs, this type of stress is usually acutely heightened when the animals are regrouped with unfamiliar individuals during the production phase (Martínez-Míró et al. 2016, review). For slaughter pigs, this regrouping usually happens after weaning and again during the fattening period and in transportation before slaughter. In these situations, the pigs need to establish a new social hierarchy, which can lead to fighting. Social stress can also be chronic, if the hierarchy within a group is not stable, an individual's social status within a group is low or the space allowance for the group is small (Martínez-Miró et al 2016, review). Limited space allowance is linked to reduced growth rate and increased frequency of aggressive behaviour (Martínez-Miró et al 2016, review). However, pigs seem to experience less social stress in larger groups (Andersen et al. 2004).

Environmental stress is caused by suboptimal housing facilities. In intensive pig farming the requirements for controlling the temperature, humidity, light, air quality (concentrations of dust and gases) are high. The optimal temperature for an animal depends on the size and production period of the pig and therefore different groups should be housed in different temperatures (Martínez-Miró et al. 2016, review). Especially in farms that are located in very hot or cold areas this can be a challenge. The important measurement for the pig is the effective temperature, which depends not only on the room temperature, but also things like ventilation, floor material and group size (Martínez-Miró et al. 2016, review). Barren housing environments with little material to perform natural behaviours are also a remarkable source of stress in modern intense pig farming (Martínez-Miró et al. 2016, review). Enriching the environment reduces harmful social behaviour, such as tail biting and manipulation of pen mates, and improves the growth rates of pigs (Mkwanazi et al. 2019). Tail biting is one of the greatest issues in the modern commercial pig farming, both in relation to economic losses and the welfare of the animals (Valros & Heinonen, 2015, review). There are several environmental factors that can contribute to the emergence of the behaviour: diet, feeding-related frustrations, pain,

poor health, genetics, high stocking density, poor microclimate conditions and the lack of manipulable materials in the surroundings (Taylor et al. 2010, review).

2.4.2 Response to stress

The stressors mentioned above are a threat to the homeostasis of the pig and they can elicit several biological responses, including behavioural, neuroendocrine and immunological responses. Normal behavioural responses to a perceived threat are avoiding or hiding from it or confronting the threat. If an animal is not able to react to a stimulus in an appropriate manner, abnormal behaviour, such as stereotypes, redirected abnormal behaviour and excessive aggressive behaviour, can occur (Matínez-Miró et al. 2016, review). A stereotype in the context of behaviour is defined as a repetitive behaviour pattern with no obvious goal or function (Mason et al. 1991, review). Stereotypes typically arise when an animal is unable to interact with its environment in a satisfying manner. Environmental factors that might lead to abnormal behaviour include restraint, lack of stimulation and unavoidable stress or fear (Mason et al. 1991, review). In pigs, common abnormal behaviour include tail and ear biting (Martínez-Míro et al. 2016, review). Besides behavioural signs, physiological measures can be used to evaluate stress in animals. Activation of the sympathetic-adrenal-medullary (SAM) and the hypothalamo-pituitary-adrenocortical (HPA) neuroendocrine axes are often used to assess stress response in animals (Martínez-Miró et al. 2016, review; Mormède et al. 2007). The activation of SAM axis occurs when the animal encounters an acutely stressful situation and results, among other things, in the release of epinephrine and norepinephrine from the adrenal medulla and glucose and lipids from the liver (Martínez-Miró et al. 2016, review). The HPA response includes the release of ACTH by the pineal gland and glucocorticoids by the adrenal cortex (Mormède et al. 2007). However, the HPA axis can respond to various excitement-inducing stimuli, such as feeding in pigs and cortisol is also influenced by the photoperiod and the time of the day, which makes cortisol an insufficient measure of stress if not used in combination with other indicators (Mkwanazi et al. 2019).

3 MATERIALS AND METHODS

Materials and methods are previously published in Larsen et al. 2019

The study was conducted in accordance with a protocol approved by the Danish Animal Experiments Inspectorate (Journal no. 2015-15-0201-00593).

3.1 Animals and housing

Three batches of pigs were included in the study conducted from 2015 to 2016 amounting to 1160 finisher pigs (595 females and 565 castrated males) in 80 study pens altogether. The pigs were crossbreeds from Yorkshire x Danish Landrace sows inseminated with Duroc boar semen. All pigs were born and weaned at the same farm. The male pigs were castrated surgically and half of the pigs also underwent tail docking within the first four days of birth. The pigs were weaned at four weeks of age and moved at five to six weeks of age to the experimental stables at Department of Animal Science, Aarhus University, Denmark.

As weaners the pigs were divided between 14 pens according to the tail type (docked or intact). Pigs were housed only with pigs of similar tail type, otherwise the pens were selected randomly. The pigs were weighed and ear marked individually a day after the arrival (average weight on arrival: 8.72 ± 2.89 kg). There were 37 weaners in each pen, equalling to 0.34m^2 floor space per pig. The floor space was divided equally between solid, drained and slatted flooring. The solid floor area had a cover as well as wood shavings as bedding material. Since the main purpose of the study was to investigate tail biting during the slaughter pig period, it was essential to avoid this behaviour in weaners. Therefore, the conditions for weaners were kept above the standard level. This included more water access points and feeding stalls than required by law per pen, ample provision of straw and provision of other enrichment materials such as paper bags, wooden blocks and hanging ropes. These additional enrichment materials were provided one type per day at presumed times of higher stress, that is at arrival and whenever the farm staff saw signs

of tail biting. The weaners were fed *ad libitum* with commercial dry feed. The pigs were housed as weaners until an average weight of approximately 30 kgs and then redistributed into study pens where they were housed until slaughter. The average weight at the beginning of the study period was 31.6 ± 6.6 kg and at the end of the study 103.3 ± 16.2 kg.

The slaughter pigs were housed in sections that each consisted of 16 2.48 x 5.45 m sized pens. The floor space was also here divided equally between drained, slatted and solid concrete flooring (Figure 1). The pens had a dry-feeder with either two or three feeding places according to the number of pigs in the pen (11 or 18 pigs, respectively; see Section 3.2). The feeder was placed above the drained floor. Further, the pens had two drinking cups (circles in Figure 1) above the slatted floor and two wooden sticks as enrichment in separate racks (blue squares in Figure 1) on the solid floor.

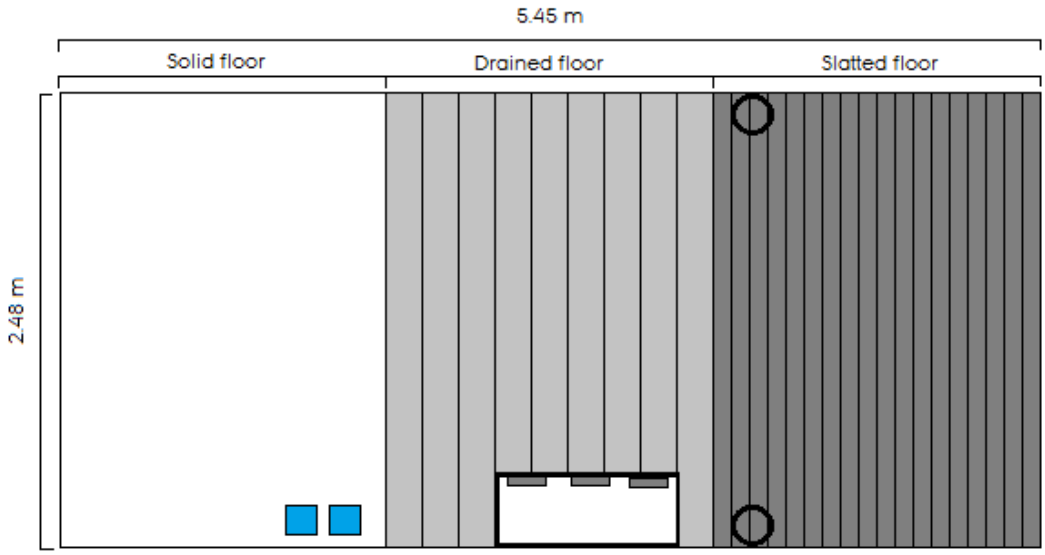

Figure 1 Pen design for pens with 18 pigs. Pens with 11 pigs had two-placed feeders, otherwise the pens had similar dimensions (Larsen et al. 2019).

The slaughter pigs were fed *ad libitum* with two commercial dry feeds (switched at a weight of approximately 50 kg) and the feeding troughs were filled three times per day at 03:00, 10:00 and 18:30 h. Electric lights were on from 05:30 to 18:30 h. Two small windows in the section doors provided artificial light. Temperature was controlled with a ventilation system (Skov A/S, Roslev, DK) that gradually decreased the temperature from

21 ºC in the beginning to 17 ºC at the end of the slaughter pig period. Each pen also had a cooling shower system above the slatted floor (Skov A/S, Roslev, DK) that was controlled automatically on room-level. It was activated from 08:00 to 20:00 h except if the outdoor temperature fell below 5 ºC. The system followed a linear curve going from 1% at a 0.5 ºC increase from the temperature curve to 100% at a 4 ºC increase. At 1%, the sprinklers were turned on with 45 minutes intervals for 1 minute and at 100% with 20 minutes intervals for 3 minutes. In the current study, the minimum was 14%.

3.2 Experimental design

Batches one and three included 32 slaughter pig pens in two sections. Batch two had 16 pens in one section due to weaner delivery problems. As part of a larger study design (Larsen et al. 2018a) and to test whether tear staining in pigs depends on different potential pen level stressors, the pens were randomly divided within each batch between one level of each of three factors: 1) tail: pigs with undocked (n=36) or docked tails (n=44), 2) straw: not provided with straw (n=40) or provided with 150 g of straw per pig per day on the solid floor (n=40) and 3) stock: stocking density of 0.73 m2/pig (n=40, 18 pigs per pen, high density) or 1.21 m2/pig (n=40, 11 pigs per pen, low density). Less pens with undocked pigs compared to pens with docked pigs could be included due to a high proportion of the undocked weaners in batch 2 arriving to the experimental stables with bleeding tails and thus could not be included in the study.

The observation and scoring of tear staining were conducted three times a week (Monday, Wednesday and Friday) each week of the study during the slaughter pig period. On the observation days all pens in the study were first observed for tail posture as a part of a larger study (Larsen et al. 2018b). This was followed by individual scorings of tail damage and tear staining. Scoring was conducted by one or two people walking in the pen, identifying a pig by the earmark, checking the tail for possible damage and giving a separate tear staining score for the left and the right eye. The tear staining protocol is shown in Figure 2 and Table 1. The protocol factors in the size of the individual pig, as the size of the tear stain is compared to the size of the eye.

Batch 1 included five different observers who were all trained according to a scoring protocol with pictures and text, both by group-discussions and practical scorings in the

stable. Batch 2 included four observers, all of whom were also included in the first batch. Batch 3 included five observers, of whom one was new and trained by the others. Batch 4 included four observers, of which one was new and trained by the others. Neither inter nor intra observer reliability was calculated.

Figure 2 The picture protocol used for scoring of tear staining (TS; DeBoer-Marchant-Forde Scale) (DeBoer et al. 2015). TS scores 0-5, from left to right, respectively.

Table 1 The protocol used for scoring of tear staining (TS; DeBoer-Marchant-Forde Scale) (Larsen et al. 2019, based on DeBoer et al. 2015).

TS score	Description
0	No sign of tear staining
1	Staining is barely detectable and does not extend below the eyelid
2	Staining is obvious and covers <50% of total eye area
3	Staining is obvious and covers 50-100% of total eye area
4	Staining is severe, covers ≥ 100% of total eye area, and does not extend below the mouth line
5	Staining is severe, covers >100% of total eye area, and extends below the mouth line

Besides the three scoring days per week described above, tail damage was scored daily from outside the pen by the stock personnel. If at least one pig in the pen was scored with a bleeding tail wound, then this pen would be recorded as a tail damage pen and the day of the first tail damage observation termed day0 for the respective pen. When recorded as a tail damage pen, the pen was observed for tail damage and tear staining for seven consecutive days. Afterwards, the pen was no longer included in the study and was not scored for either tear staining or tail damage.

3.3 Statistical analysis

In the initial investigation of the data it was discovered that the data included only few observations of the lowest and highest scores (332 TS score 0 and 131 TS score 5 out of the 26814 individual TS score observations). It was therefore deemed appropriate to combine TS score 0 with TS score 1 and TS score 5 with TS score 4. It was also noted that the individual TS scores could drop from a high TS score to a low TS and back to a high TS score again in the span of only three observations. This may be explained by the TS being washed off a pig due to the activation of the shower system. Therefore, data were aggregated to only include the maximum individual TS score within each week of the study. To study the TS scores separately, the scores were transformed to binomial variables (either occurring or not) for the single pig in each week of the study.

All statistical analyses were performed in R Version 3.4.3 (R Core Team, 2017) using the package ”lme4” (Bates et al. 2015) for generalised linear mixed models. All models were logistic regression using the function “glmer” with family set to binomial and were reduced according to a 5% significant level ($P<0.05$). Results are presented as the probability of each TS score and differences as odds ratios (OR) with connected 95% confidence intervals (CI).

To test the effect of week (1 to 9, continuous), eye (left v. right), tail (docked v. undocked), straw (yes v. no) and stock (low v. high stocking density) on the probability of each TS score, the data were aggregated to pen level. Also, only the pens that were never scored as a tail damage pen were included to have data where all pens were represented in all weeks of the study. Thus, each observation contained information on the total number of pigs in the pen and the number of observations of each TS score within the pen each week. These data included 315 observations of each TS score for both the left and right eye. Four models were created, one for each TS score, all including the same main effects, interactions and random effects. The models included the main effects week, eye, tail, straw and stock, all interactions between week and the other main effects, and all interactions between eye and the remaining main effects. Lastly, the model specified

a random intercept and slope (for the main effects week and eye) for each pen nested within batch number (1-3).

To test the effect of sex and average daily gain (ADG) from the beginning to the end of the study on the probability of each TS score, the data on pig level were further aggregated to include only one observation per individual pig for the entire study period. Again, only the pens that were never scored as a tail damage pen were included to have data where all pens were represented in all weeks of the study. Thus, each observation included the number of observation weeks and the number of each TS score for each pig. These data included 490 observations of each TS score for both the left and right eye. The models were created separately for the left and right eye. However, since the previous analysis (presented above) showed that eye did not have an effect on TS score 1 and 3, this analysis was only performed on left eye data for these TS scores whereas the analysis was performed on both left and right eye data for TS score 2 and 4. In total, six models were created, all including the same main effects, interactions and random effects. The models included the main effects sex and ADG, the interaction between the two, and the individual assignment weight as a covariate. Further, the model specified a random intercept for each pen nested within batch number (1-3).

To test whether the probability of each TS score changed prior to the scoring of tail damage on day0 and whether this was different for pens not scored with tail damage, each tail damage pen was paired with control pens from the same batch with the same treatment level of tail, straw and stock. The pen level data were further aggregated to only include the last three observation days (one week) prior to day0 for each respective pair of tail damage and control pens (21 pairs in total). In this process, a day category variable relative to day0 with three levels were created: day1-3 (1-3 days prior to day0), day4-5, and day6-7. These data included 192 observations of each TS score for both the left and right eye. The models were created separately for the left and right eye. Again, only left eye data were used for TS score 1 and 3 and both left and right eye data were used for TS score 2 and 4. In total, six models were created, all including the same main effects, interactions and random effects. The models included the main effects pen type (tail damage v. control), day category (day1-3 v. day4-5 v. day6-7) and period (1: week 1-3; 2: week 4-6; 3: week 7-9) and the interactions between pen type and the remaining main

effects. Further, the model specified a random intercept for each pen nested within pair number (1-21) and batch number (1-3). The model on TS score 1 further included the main effect tail and the interaction between pen type and tail, since tail was shown in a previous model to affect the probability of TS score 1.

4 RESULTS

Results are previously published in Larsen et al. 2019

4.1. Descriptive development and variation

All four TS categories were represented in all weeks of the study, also when only the highest individual weekly scores were considered. The means of the highest individual TS scores for each week seem to increase with weeks into the study period. The development is similar with both eyes. The deviation in the mean highest TS scores is quite stable, both overall (ranging from 0.70 to 0.86 for the left eye and from 0.70 to 0.89 for the right) and within-pen (from 0.66 to 0.82 for the left eye and from 0.66 to 0.86 for the right). Detailed results can be seen in Table 2.

Table 2. The descriptive development and deviation in the weekly max individual tear staining (TS) score over the 9 weeks of the study period divided between the left and right eye (Larsen et al. 2019)

Weekly max TS score	Week in the study period								
	1	2	3	4	5	6	7	8	9
Left eye									
Mean	2.23	2.49	2.57	2.81	2.99	3.10	3.23	3.25	3.37
Overall SD	0.70	0.82	0.85	0.86	0.82	0.81	0.76	0.80	0.74
Within-pen SD	0.66	0.79	0.82	0.80	0.76	0.76	0.74	0.77	0.70
Right eye									
Mean	2.31	2.59	2.68	2.84	3.10	3.16	3.28	3.29	3.45
Overall SD	0.73	0.81	0.89	0.87	0.83	0.82	0.77	0.76	0.70
Within-pen SD	0.68	0.78	0.86	0.81	0.77	0.77	0.72	0.73	0.66

4.2. Week, eye, pen-level treatments and sex

4.2.1 Week

The probability of TS score 1 ($P<0.01$) and TS score 2 ($P<0.001$) decreased while the probability of TS score 4 increased ($P<0.01$) with weeks into the study. There was no significant development of TS score 3 with weeks in the study period. The results are illustrated in Figure 2.

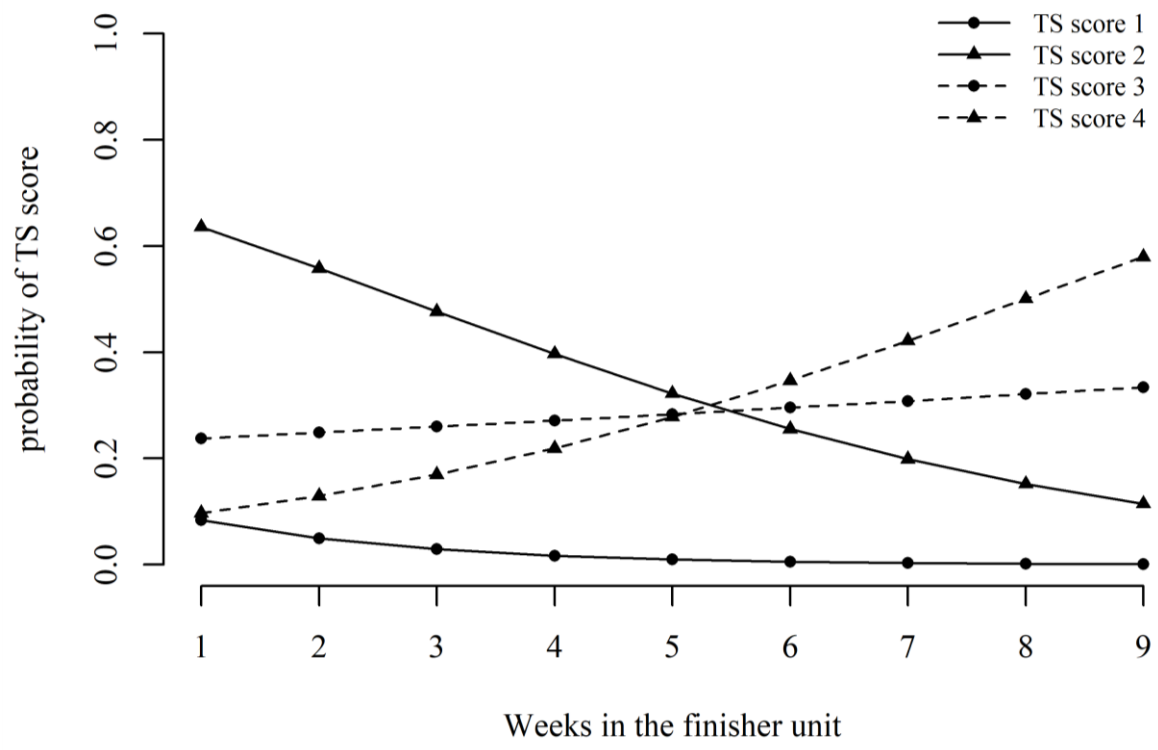

Figure 3. The development in probability of the four tear staining (TS) scores with weeks into the study period (Larsen et al. 2019).

4.2.2 Eye

There was no difference in TS score 1 or TS score 3 between the left and the right eye. For TS2, there was a higher probability on the left compared to the right eye (OR = 1.14, 95% CI [1.04, 1.26]; $P<0.05$). For TS score 4, there was a higher probability on the right eye compared to the left eye (OR = 1.22, 95% CI [1.09, 1.35]; $P<0.05$).

4.2.3 Tail

Pens with pigs that had docked tails had a higher probability of TS score 1 compared to pens with undocked pigs (OR = 1.79, 95% CI [1.02, 3.12]; $P<0.05$). No difference was found in any of the other TS categories.

4.2.4 Straw and stock

There were no differences in any of the TS categories between pens provided with straw and pens not provided with straw or between pens of low stocking density and high stocking density.

4.2.5 Sex

There were no differences between barrows and females in any of the TS categories.

4.3. Average daily gain

The probability of TS score 1 (P<0.001) and TS score 2 (P<0.001) decreased and the probability of TS score 4 increased (P<0.001) with rising ADG. TS score 3 showed no relationship with ADG. The results are illustrated in Figure 3.

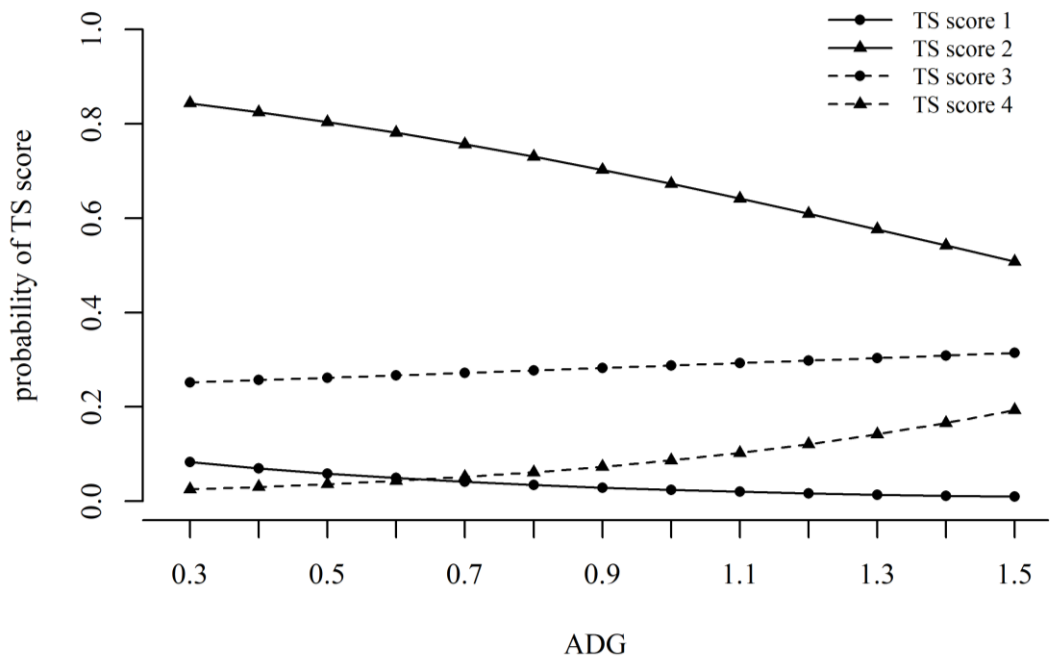

Figure 4. The development in probability of the four tear staining (TS) scores with increasing average daily gain (ADG, kg/day) (Larsen et al. 2019).

4.4. Tear staining prior to tail damage

The probability of TS score 1 was across all three day categories lower in the tail damage pens compared to their control pens (OR = 0.56, 95% CI [0.41, 0.75]; P<0.01). TS score 1 also decreased in both tail damage pens and control pens prior to day0: Probability of TS score 1 was lower on day1-3 compared to day4-5 (OR = 0.70, 95% CI [0.52, 0.94]) and day6-7 (OR = 0.65, 95% CI [0.48, 0.87]; P<0.01). TS score 4 increased in both tail

damage and control pens prior to day0: Probability of TS score 4 was lower on day6-7 compared to day4-5 (OR = 0.72, 95% CI [0.53, 0.99]; $P<0.01$) and day1-3 (OR = 0.64, 95% CI [0.45, 0.89]; $P<0.05$). The increase in TS score 4 was only found on the left eye. No effects were found on either TS score 2 or TS score 3.

5 DISCUSSION

5.1 Overall development and average daily growth

The overall development of the TS scores over the study period shows that TS in general gets more severe during the slaughter pig period. The probability of the lower scores decreases and the probability of the higher scores increases with weeks into the study period. There is also a numerical increase in the mean TS score. This general tendency was observed in both eyes. In the beginning, the most probable TS score is 2, and, while the probability of TS score 2 remains the highest until week six, it does decrease with time quite steadily. The relative number of observations of TS score 1 is the second highest in the beginning of the study, but quickly decreases: From week 4 onwards it is the least probable score and remains as such until the end of the study period. On and after week six TS score 3 and TS score 4 are more probable than lower scores.

The degree of TS also increased with ADG. The probability of the lower scores decreased and the probability of the higher scores increased the higher the ADG. As the TS scoring system does account for the size of the eye on an individual, the increase in TS should not only be due to bigger animals producing more secretion. The increasing TS both temporally and in relation to the ADG could suggest that the lacrimation system and the HG are still going through significant development in this slaughter pig phase. Telkänranta et al. (2016) observed that suckling piglets have very low TS scores, which agrees with the results of the current study. The increasing TS scores could therefore be due to either maturation of the physiology behind TS or changes in the environment over the production process that result in TS. It could be that the pigs experienced more stress with weeks into the study period, as they spent more and more time in a possibly constant stressful environment. The porphyrin content of the HG has been shown to increase with age in rats (Rodríguez et al. 1992), which could indicate that the more severe TS is due

to higher secretion of porphyrins into the lacrimal fluid. In rats, food restriction prevents the age-associated rise (Rodríguez et al. 1992), and if this applies to pigs as well, it could be that the reason for lower TS scores with lower ADG is lower food intake. In rats, there is some evidence (Harkness & Ridgway 1980) that larger individuals react to stressors by exhibiting chromodacryorrhoea faster than smaller animals, but the relation between weight and the degree of TS has not been established in rats. However, this further supports the relation between TS and weight. It also raises the question whether pigs with higher ADG could have higher TS scores because their stress response elicits higher degree of TS compared to pigs with lower ADG, even though the perceived stressor was equal.

The relation between TS and ADG could diminish the potential of using TS as a marker of stress in the pig production, since the negative relation between growth rate and stress in pigs is quite well established (Martínez-Miró et al. 2016, review). In the rat and the hamster, changes in the level of thyroid hormones change the activity of the HG, therefore growth-related thyroid hormones may affect TS as well (Hoffman et al. 1990, Baccari et al. 2004, Monteforte et al. 2008). The positive relation between ADG and TS may therefore also be due to hormonal differences between pigs with different growth rates. Overall, the age and the growth of the pig is an important factor in the development of TS. This should be considered if TS is utilized in farm conditions as it might not indicate similar things throughout all production phases. Additionally, because the scores were overall high towards the end of the study, individual differences are probably harder to observe later in the slaughter pig period than in the beginning where only a few pigs exhibited higher TS scores. Thus, TS as a tool might not be at its most sensitive towards the end of the slaughter pig period.

5.2 Effect of pen-level stressors

There was no significant difference in TS found between pens that were given straw as additional enrichment and those that only had wooden sticks as chewing materials. In previous studies (Telkänranta et al 2016, DeBoer et al. 2015) lower TS scores were found with pigs that were provided with more interesting enrichment or housed in a minimally enriched environment compared to a barren environment. Straw in general has been found

to be an effective enrichment material (Mkwanazi et al. 2019, review). Thus, it could be expected that TS would be less severe in pens that were provided with additional straw enrichment also in the current study. Straw provision did decrease the likelihood of tail damage (Larsen et al. 2018a), so it seems that the additional enrichment was remarkable enough to affect the behaviour of the pigs. Perhaps there was another stressor, such as feeding-related competition, that overrode the effect of enrichment on TS.

There was also no difference in development of TS between pens with low stocking density and high stocking density. It could be expected that the high stocking density would be more stressful and that the effect would be more effective as the pigs grew in size, decreasing the space allowance further. However, pigs stocked with smaller density also had a smaller group size (11 vs. 18 individuals) and small group size may cause increased aggressive behaviour and hence higher social stress (Andersen et al. 2004), which could affect the TS scores. It is also possible that the difference in stocking density was not large enough to show differences in TS. The lack of effect might, again, also have been influenced by stressors common to all pens, such as microclimate conditions, noise and competition at the feeder (Martínez-Miró et al. 2016, review).

Pens that had docked tails had a higher probability of TS score 1 compared to undocked pens pigs. This could suggest that docked pigs experienced less stress, perhaps due to less tail biting and tail damage (Larsen et al. 2018a). However, because there was no difference in other TS categories, this finding could be incidental.

5.3 Effect of sex

No difference in TS scores was found between male and female pigs in the current study. While the miniature pig HG shows marked sexual dimorphism in its histology, it is not known whether this difference would manifest in different TS scores. It should also be noted that the castration of the male pig might diminish or undo the difference between genders, similarly to the feminization of the castrated male hamster HG (Buzzell 1996). All the male pigs in this study were castrated as sucklers and therefore there is no evidence available whether the TS patterns would be different in intact males. Hypothetically, the castration of males, or rather, the lack of boars, might affect also the TS of female pigs,

as it is well established that the presence of a boar affects the hormone production of female pigs and advances the start of puberty in gilts (Pearce & Hughes 1985). Studying the TS in sows, especially in the presence of boars and during heat, could give further insight into the hormonal background of the regulation of TS in pigs. Observing the TS patterns of boars and sows around heat time is also interesting in the light of the HG being a possible source of pheromones (Payne 1994, review).

5.4 Tear staining prior to tail biting

The probability of TS score 1 in a pen decreased before a tail damage incident (day0). The probability of TS score 4 increased, and this was only seen on the left eye. However, the changes prior to a tail damage incident were seen in control pens as well. Therefore, while increasing TS was not a good detector of which pens would go through a tail damage incident in the current study, it could have been an indicator for an increasing effect of a stressor common to both pen types, such as temperature or air quality. That the increase prior to tail damage was only found for the left eye aligns with the findings on previous studies (Telkänranta et al. 2016, DeBoer et al. 2015), as stressors or negative welfare parameters have been found to have a stronger relation with the left than the right eye TS. The majority of evidence on emotional cerebral lateralisation from studies in non-human vertebrates suggests right hemispheric dominance in fear and aggression (Leliveld et al. 2013). As the left eye is connected to the right hemisphere, this could be a possible explanation for the difference between the eyes and a further link between TS and negative emotional state. The relation between lateralisation and stress in pigs, however, is not straightforward: It seems that different hemispheres may dominate in processing different types of stress (Leliveld 2019).

5.5 Limitations

There were some factors that could have diminished the reliability of the study. Some concern among the observers was raised during the study that the cleanliness of the pens could affect the scoring of TS. It was naturally harder to evaluate TS in a pig that had significant amounts of dirt on the face. Provision of straw could enhance rooting behaviour and possibly also increase the dirtiness on the face area. Another remark made

by the observers was that the colour and intensity of the tear stains could vary from very dark brown, thick secretion to light, runny secretion which was sometimes quite hard to observe. This difference was seen between individuals, but also between different observations on the same animal. This could be due to different relative secretion levels between the orbital glands at different times, or perhaps different secretory states of the HG. It is unclear whether the colour and consistency of the secretion have any significance in relation to TS as an indicator of stress in pigs. The effect of the cooling showers on the consistency of the secretions is also unknown. The subjective opinions of the observes carrying out the study at the stables was that TS score 4 (and TS score 5) may have been the most reliably scored as the large stains were easy to observe and the category cut-off was clear (over 100% of the size of the eye). TS score 3 was perhaps the most difficult to differentiate from surrounding categories (TS score 2 and TS score 4) on stable conditions.

6 CONCLUSIONS

The severity of TS consistently increased over the study period. This suggests that either the orbital glands are still going through a maturation process over the slaughter pig period, or that the environment is increasingly or cumulatively stressful for the pig. TS also correlated with ADG. This also connects TS with the development of the pig. The probability of TS score 1 decreased and TS score 4 increased before a tail damage incident, but this was only seen on the left eye and was also seen in the control pens. Therefore, TS did not work as an early indicator of a tail damage incident but may have been detecting increasing stress level by a factor common to both pen types. The relationship between TS and ADG as well as TS and age should be considered if TS scoring is utilised as a stress indicator. Furthermore, additional studies are needed on the timeline of individual development of TS after being exposed to a stressor as well as on the different types of stressors that may elicit TS as a response, to better establish the connection between stress and TS in pigs.

7 ACKNOWLEDGEMENTS

I take this opportunity to sincerely thank my director Anna Valros for her professional guidance, mentorship and never-ending patience through the whole process. Without her I would not have had the chance to participate in this research project and create the many unforgettable memories and friendships that came with the experience.

I would also like to express my profound gratitude to my supervisor Mona Lilian Vestbjerg Larsen for kindly welcoming me into her project as well as her country, for her invaluable work with the statistics and for all the support both academic and practical.

Last, I want to thank my friends, family and work colleagues for all their words and actions of encouragement.

8 REFERENCES

Abd EA, Abou-Elhamd A, Hassan A. Effects of Administration of Melatonin on the Harderian Gland of Sheep. J Interdiscip Histopathol 2014, 2: 19-25.

Akers RM, Denbow DM. Special Senses. In Akers RM, Denbow DM (eds.) Anatomy and Physiology of Domestic Animals. 2nd ed. Wiley-Blackwell, Ames, Iowa 2013: 97-323.

Andersen IL, Naevdal E, Bakken M, Boe KE. Aggression and group size in domesticated pigs, Sus scrofa: 'When the winner takes it all and the loser is standing small' Anim Behav 2004, 68: 965–75.

Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J Stat Softw 2015, 67: 1-48.

Baumans V. Methods for evaluation of laboratory animal well-being Altern Lab Anim. 2004, 32 Suppl 1A: 161-162.

Burn CC, Deacon RM, Mason GJ. Marked for life? Effects of early cage-cleaning frequency, delivery batch, and identification tail-marking on rat anxiety profiles. Dev Psychobiol 2008, 50: 266-277.

Buzzell G. Sexual dimorphism in the harderian gland of the Syrian hamster is controlled and maintained by hormones, despite seasonal fluctuations in hormone levels: Functional implications. Microsc Res Tech 1996, 34(2): 133-138.

Cabral VP, Laus JL, Dagli MLZ, Pereira GT, Talieri IC, Monteiro ER, Mamede FV. Canine lacrimal and third eyelid superficial glands' macroscopic and morphometric characteristics. Cienc Rural 2005, 35(2): 391-397.

Chieffi G, Baccari GC, Monteforte R, de Lange P, Raucci F, Farina P, Lanni A. Thyroid hormone affects secretory activity and uncoupling protein-3 expression in rat harderian gland. Endocrinology 2004, 145(7): 3338-3345.

Chieffi G, Baccari GC, Di Matteo L, D'Istria M, Minucci S, Varriale B. Cell biology of the harderian gland. Int Rev Cytol 1996, 168: 1-80.

Dartt DA. Control of mucin production by ocular surface epithelial cells. Exp Eye Res, 2004, 78(2): 173-185.

Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. Prog Retin Eye Res 2009, 28(3): 155–177.

DeBoer S, Garner J, McCain R, Lay Jr D, Eicher S, Marchant-Forde J. An initial investigation into the effects of isolation and enrichment on the welfare of laboratory pigs housed in the PigTurn® system, assessed using tear staining, behaviour, physiology and haematology. Anim Welf 2015, 24(1): 15-27.

DeBoer S, Marchant-Forde J. Tear staining as a potential welfare indicator in pigs. In the Proceedings of the 47th Congress of the International Society for Applied Ethology 2013: 125, Florianopolis, Brazil.

Done S, Williamson SM, Strugnell BW. Body systems: Nervous and locomotor system. In Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW (eds.). Diseases of swine. 10th ed. Wiley-Blackwell, US 2012: 294-328.

Drummond JG, Curtis SE, Simon J, Norton HW. Effects of aerial ammonia on growth and health of young pigs. J Anim Sci 1980, 50(6): 1085-1091.

Duncanson GR. Veterinary treatment of pigs. 2013 CABI Publishing, Wallingford, Oxon.

Elgayar SAM, Abou-Elghait AT, Sayed AA. Morphology of female guinea pig harderian gland during postnatal development - secretory endpieces. Eur J Anat 2015, 19: 15-26.

Evans BA, Donnelly TM. Chromodacryorrhea, Diseases and disorders, small mammals: Rats. In Mayer J, Donnelly TM (eds.). Clinical veterinary advisor - birds and exotic pets. Saint Louis: W.B. Saunders. 2013: 242-252.

Gancharova OS, Manskikh VN. Age-related changes in the rat lacrimal gland: Specific morphology and unknown nature. Russian Journal of Developmental Biology 2014, 45(5): 335-242.

Gelatt, KN. Surgery of nasolacrimal apparatus and tear systems. In Gelatt KN, Gelatt JP (eds.). Veterinary ophthalmic surgery. Edinburgh, W.B. Saunders, 2011: 141-156.

Glenwood GG, MacKay EO. Chapter 3: Physiology of the Eye in Gelatt KN, Gilger BC, Kern TJ. (eds.) Veterinary Ophthalmology. 5th ed. Wiley-Blackwell, 2013.

Gruber T. Housing, feeding, hygienic conditions, health and management of finishing pigs in organic farming units. PhD thesis, University of Veterinary Medicine, Vienna, Austria 2002.

Harkness JE, Ridgway MD. Chromodacryorrhea in laboratory rats (rattus norvegicus): Etiologic considerations. Lab Anim Sci 1980, 30(5): 841-844.

Henker R, Scholz M, Gaffling S, Asano N, Hampel U, Garreis F, Hornegger J, Paulsen F. Morphological features of the porcine lacrimal gland and its compatibility for human lacrimal gland xenografting. PLoS One, 2013: 12;8(9): e74046.

Hoffman RA, Habeeb P, Buzzell GR. Further studies on the regulation of the harderian glands of golden hamsters by the thyroid gland. J Comp Physiol 1990, 160(3): 269-275.

Hussein OA, Elgamal DA, Elgayar SAM. Structure of the secretory cells of male and female adult guinea pigs harderian gland. Tissue Cell 2015, 47(3): 323-335.

Ibrahim ZH, Abdalla AB, Osman DI. A gross anatomical study of the lacrimal apparatus of the camel (Camelus dromedaries). J Sc Tech 2006, 9: 1-8.

Junquira LC, Carneiro J. Basic histology. 10th ed. McGraw- Hill Companies, USA 2003.

Kerins CA, Carlson DS, McIntosh JE, Bellinger LL. Meal pattern changes associated with temporomandibular joint inflammation/pain in rats; analgesic effects. Pharmacol Biochem Behav, 2003, 75(1): 181-189.

Larsen MLV, Andersen HM-L, Pedersen LJ. Which is the most preventive measure against tail damage in finisher pigs: Tail docking, straw provision or lowered stocking density? Animal 2018: 12(6), 1260-1267.

Larsen MLV, Andersen HM-L, Pedersen LJ. Tail posture as a detector of tail damage and an early detector of tail biting in finishing pigs. Appl Anim Behav Sci 2018, 209: 30-35.

Larsen MLV, Gustafsson A, Marchant-Forde J, Valros A. Tear staining in finisher pigs and its relation to age, growth, sex and potential pen level stressors. Animal 2019: 13(8), 1704-1711.

Leliveld LMC, Langbein J, Puppe B. The emergence of emotional lateralization: evidence in non-human vertebrates and implications for farm animals. Appl Anim Behav Sci 2013, 145: 1-14.

Leliveld LMC. From Science to Practice: A Review of Laterality Research on Ungulate Livestock. Symmetry, 2019, 11(9): 1157.

López, J. M., Tolivia, J. F., & Alvarez-Uria, M. Postnatal development of the harderian gland in the syrian golden hamster (mesocricetus auratus): A light and electron microscopic study. The Anatomical Record 1992, 233(4): 597-616.

Marchant-Forde AEM and Marchant-Forde JN (2014) Social status and tear staining in nursery pigs. In: Manteca X, Estevez I, Marin RH and Averos X (eds.) Proceedings of 48th Congress of the International Society for Applied Ethology p 145. 29 July-2 August 2014, Vitoria-Gasteiz, Spain.

Martínez-Miró S, Tecles F, Ramón M, et al. (2016). Causes, consequences and biomarkers of stress in swine: an update. BMC Vet Res 2016, 12(1): 171.

Mason G. Stereotypies: A Critical Review. Anim Behav 1991, 41: 1015–1037.

Mason G, Wilson D, Hampton C, Würbel H. Non-invasively Assessing Disturbance and Stress in Laboratory Rats by Scoring Chromodacryorrhoea. Altern Lab Anim 2004, 32(Suppl 1A): 153–159.

Meng ID, Kurose M. The role of corneal afferent neurons in regulating tears under normal and dry eye conditions. Exp Eye Res 2013, 117: 79-87.

Mkwanazi MV, Ncobela CN, Kanengoni AT, Chimonyo M. Effects of environmental enrichment on behaviour, physiology and performance of pigs - A review. Asian-Australas J Anim Sci 2019, 32(1): 1-13.

Monteforte R, Santillo A, Lanni A, D'Aniello S, Baccari GC. Morphological and biochemical changes in the harderian gland of hypothyroid rats. J Exp Biol 2008, 211(4): 606-612.

Mormède P, Andanson S, Aupérin B, Beerda B, Guémené D, Malmkvist J, Manteca X, Manteuffel G, Prunet P, van Reenen CG, Richard S, Veissier I. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol Behav 2007, 92(3): 317-339.

Munkeby BH, Smith HJ, Winther-Larssen EH, Bjørnerud A, Bjerkås I. Magnetic resonance imaging of the harderian gland in piglets. J Anat 2006, 209(5): 699-705.

Oliveira CA, Telles LF, Oliveira AG, Kalapothakis E, Gonçalves-Dornelas H, Mahecha GAB. Expression of different classes of immunoglobulin in intraepithelial plasma cells of the harderian gland of domestic ducks anas platyrhynchos. Vet Immunol Immunopathol 2006, 113(3–4): 257-266.

O'Malley C, Turner S, D'eath R, Steibel J, Bates R, Ernst C, Siegford J. (2019). Animal personality in the management and welfare of pigs. Appl Anim Behav Sci 2019, 218: 104821.

Ortiz GG, Feria-Velasco A, Falcón-Franco MA, Bitzer-Quintero OK, García JJ, Rosales A, Reiter RJ. Different patterns in the histology and autofluorescence of the harderian glands of the syrian hamster, rat, mouse, mongolian gerbil and guinea pig. Anat Histol Embryol 2001, 30(2): 107-115.

Payne AP. The harderian gland: A tercentennial review. J Anat 1994, 185: 1-49.

Payne AP. The harderian gland: Its biology and role in porphyrin synthesis. Mol Aspects Med 1990, 11(1): 145-152.

Pearce GP, Hughes PE. The influence of daily movement of gilts and the environment in which boar exposure occurs on the efficacy of boar-induced precocious puberty in the gilt. Animal Production 1985, 40(1): 161-167.

Pinard CL, Weiss ML, Brightman AH, Fenwick BW, Davidson HJ. Normal anatomical and histochemical characteristics of the lacrimal glands in the American bison and cattle. Anat Histol Embryol 2003, 32: 257–262.

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing 2017, Vienna, Austria. URL https://www. R-project.org/.

Rehorek SJ, Hillenius WJ, Sanjur J, Chapman NG. One gland, two lobes: Organogenesis of the "harderian" and "nictitans" glands of the chinese muntjac (muntiacus reevesi) and fallow deer (dama dama). Ann Anat 2007, 189(5): 434-446.

Rehorek SJ, Holland JR, Johnson JL, Caprez, JM, Cray J, Mooney MP, Smith TD. Development of the Lacrimal Apparatus in the Rabbit (Oryctolagus cuniculus) and Its Potential Role as an Animal Model for Humans. Anat Res Int 2011, 2011: 623186.

Remington LA. Chapter 14: Autonomic Innervation of Ocular Structures. In Remington LA (ed.) Clinical Anatomy and Physiology of the Visual System. 3rd ed. Butterworth-Heinemann, 2012: 253-272.

Rodríguez C, Menéndez-Peláez A, Howes KA, Reiter RJ. Age and food restriction alter the porphyrin concentration and mRNA levels for 5-aminolevulinate synthase in rat Harderian gland. Life Sci 1992, 51: 1891-1897.

Rodríguez-Colunga MJ, Rodríguez C, Antolín I, Uría H, Tolivia D, Vaughan MK, Menéndez-Peláez A. Development and androgen regulation of the secretory cell types of the Syrian hamster (Mesocricetus auratus) Harderian gland. Cell Tissue Res 1993, 274: 189.

Santos R, Carlini EA. Red Lacrimal Secretion (Chromodacryorrhea) Induced by Cholinergic Drugs in Rats Subjected to the Watertank Technique. Pharmacology 1988, 36: 61-68.

Sbarbati A, Calderan L, Nicolato E, Marzola P, Lunati E, Donatella B, Osculati F. Magnetic resonance imaging of the rat harderian gland. J Anat 2002, 201(3): 231-238.

Sinisalo A, Niemi JK, Valros A, Heinonen M. Tail biting and production performance in fattening pigs. Livest Sci 2012, 14: 220–225.

Taylor NR, Main DCJ, Mendl M, Edwards SA. Tail-biting: A new perspective. Vet J 2010, 186(2): 137-147.

Telkänranta H, Marchant-Forde JN, Valros A. Tear staining in pigs: a potential tool for welfare assessment on commercial farms. Animal 2016, 10: 318–325.

Valros A, Ahlstrom S, Rintala H, Hakkinen T, Saloniemi H. The prevalence of tail damage in slaughter pigs in Finland and associations to carcass condemnations. Acta Agr Scand A-An 2004, 54: 213–219.

Valros A Heinonen M. Save the pig tail. Porc Health Manag 2015, 1: 2.